

PCT

WORLD INTELLECTUAL PROPERTY
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER

(51) International Patent Classification⁶:

A61K 31/55, C07D 281/10

A1

(11) In

WO 9607415A1

(43) International Publication Date:

14 March 1996 (14.03.96)

(21) International Application Number: PCT/CA95/00514

(22) International Filing Date: 11 September 1995 (11.09.95)

(30) Priority Data:

08/303,826

9 September 1994 (09.09.94)

US

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(CA).(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH,
CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE,
KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN,
MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK,
TJ, TM, TT, UA, UG, UZ, VN, European patent (AT, BE,
CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT,
SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML,
MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ,
UG).

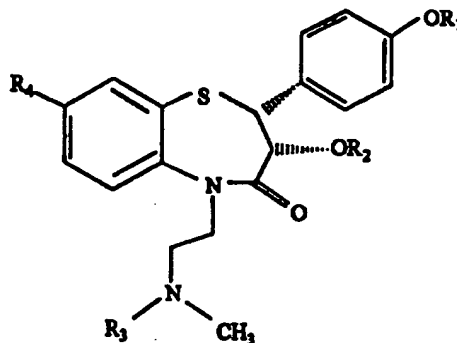
Published

*With international search report.**Before the expiration of the time limit for amending the
claims and to be republished in the event of the receipt of
amendments.*

(54) Title: MYOCARDIAL PROTECTION USING BENZOTHIAZEPINONES

(57) Abstract

Myocardial protection is achieved by administering to a patient an effective amount of a benzothiazepin derivative. The benzothiazepin derivatives have general chemical formula (I), wherein R_1 represents hydrogen or methyl, R_2 represents hydrogen or acetyl, R_3 represents hydrogen or methyl, and R_4 represents hydrogen or a halogen, with the proviso that R_1 cannot be methyl when R_2 = acetyl, R_3 = methyl and R_4 = hydrogen. The above compounds may also be used in other clinical conditions such as treatment of stable and unstable angina, non-Q-wave myocardial infarction, cerebral trauma ischemia and reperfusion, organ graft preservation, etc.



(I)

(2R,3R)

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MYOCARDIAL PROTECTION USING BENZOTHIAZEPINONES

TECHNICAL FIELD

5 The present invention relates to a method of providing myocardial protection in a patient during ischemia and reperfusion, utilizing a benzothiazepin derivative.

BACKGROUND ART

10 Timely reperfusion of ischemic myocardium limits infarct size in the experimental animal, and clinical data collected over the past decade with thrombolytic therapy have documented the benefits of early reperfusion on myocardial damage, ventricular function and mortality in patients with acute myocardial infarction (1,2).

15 Myocardial salvage can however be compromised by such complications as coronary re occlusion and severe residual coronary stenosis (11). Furthermore, results of animal studies and clinical investigations have provided convincing evidences that even when vessel patency is achieved, damage still proceeds in the post-ischemic myocardium (3,4). Although it is not yet unequivocally established that
20 reperfusion can damage normal tissue, studies have shown that reperfusion not only accelerates death of irreversibly injured myocardium, but may also compromise survival of jeopardized, but still viable myocytes salvaged by reperfusion. These so-called reperfusion injuries may represent more than 50% of ultimate infarct size (5).
25 Development of adjuvant treatments to protect the post-ischemic myocardium and maximize benefits of coronary reperfusion has thus become a major target of modern cardiovascular research.

30 With tissue re oxygenation, calcium-dependent mechanisms can mediate tissue damage through generation of oxygen derived radicals. A significant source of free radicals is the neutrophil which also releases lysosomal enzymes mediating cell destruction and vessel damage. Neutrophils and platelets accumulate in the reperfused territory and particularly at the edge of the infarct and the salvaged myocardium (5, 8, 11). These inflammatory cells accumulate at the wrong place and
35 at the wrong time compromising recovery of reversibly injured myocardium salvaged by early reperfusion.

In the past years, it has been demonstrated that some benzothiazepin calcium antagonists given at the time of reperfusion, at therapeutic doses, after coronary occlusion, protect the reperfused myocardium, reducing infarct size permanently by more than 40% (6-8).

5 These conditions and observations could not be met or reproduced by some other calcium antagonists, suggesting that additional effects than blockade of slow-L calcium channels is likely involved in the protective mechanism. The cardio-protection was observed at dosages having no effect on cardiac work load, as estimated
10 by the pressure rate product, and was accompanied by improvement of the no-reflow or the circulatory failure developing in the reperfused ischemic myocardium, at doses having no measurable direct coronary vasodilating effects. Finally, it was demonstrated that these agents were preventing neutrophil accumulation in the post-ischemic myocardium.

15 The results led to the conclusion that some calcium antagonists could be useful in patients with acute myocardial infarction undergoing thrombolytic therapy. However, recent clinical trials have documented the risks and benefits of calcium antagonists in post-myocardial infarction patients (9, 10). Those acting on afterload are not
20 recommended and others like diltiazem offer protection against reinfarction only in subgroups of patients. Considering the above data, Applicant searched for benzothiazepin derivatives with cardioprotective effects and possessing no cardiodepressive nor hypotensive effects that could limit their use in the set up of acute myocardial infarction and
25 thrombolytic therapy.

It is an object of the present invention to provide a substance which can reduce infarct size at doses having no significant direct vasoactive action.

30 It is another object of the present invention to provide a method of ensuring myocardial protection in a patient during ischemia and reperfusion.

DISCLOSURE OF INVENTION

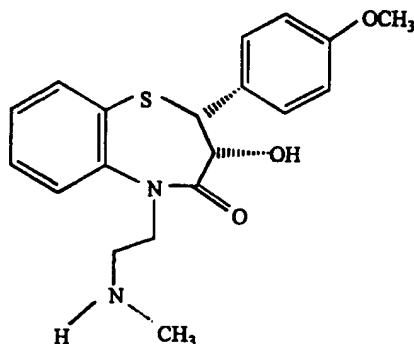
35 These and other objects of the invention may be achieved in a method of providing myocardial protection in a patient during ischemia and reperfusion, which comprises administering to said patient an effective amount of (2R,3R)-2,3-dihydro-3-hydroxy-2-(4-

methoxyphenyl)-5-[2-(methylamino)-ethyl]-1,5-benzothiazepin-4(5H)one or derivatives thereof. For the sake of brevity and convenience, the above benzothiazepin derivative or a salt thereof will be referred to in the present description as protectazem.

5 Preferably, protectazem salts or derivatives thereof are administered, most advantageously by intravenous injection, after coronary occlusion up to a few minutes before coronary reperfusion, during reperfusion, and potentially before ischemia.

10 In accordance with another embodiment of the invention, protectazem is administered at doses which vary between about 20 and about 200 mg or more depending on body weight adjustments and future data from pharmacological and pharmacodynamic studies in humans.

15 The compound protectazem which stands for (2R,3R)-2,3-dihydro-3-hydroxy-2-(4-methoxyphenyl)-5-[2-(methylamino)ethyl]-1,5-benzothiazepin-4(5H)-one has been documented as Registry No. 86408-40-4 but its synthesis and physical characteristics have not been reported. The compound may be represented by the following structural formula:



20

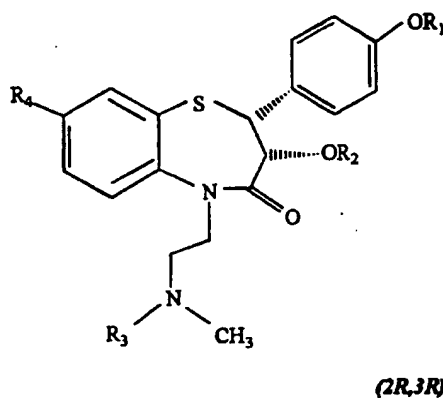
(2R,3R)

This compound is now characterized as its hydrochloride by the following: m.p. 208-209°C; $[\alpha]_{D23} - 122.8^\circ$ (0.5, H₂O).

25 In accordance with the invention, protectazem may be prepared by condensing *trans* methyl 3-(4-methoxyphenyl)glycidate with 2-aminothiophenol to give the compound methyl 3-[(2-aminophenyl)thio]-2-hydroxy-3-(4-methoxyphenyl) propionate in its racemic form, saponifying the latter with methanolic aqueous sodium

hydroxide to give the corresponding racemic acid 3-[(2-aminophenyl)thio]-2-hydroxy-3-(4-methoxyphenyl) propionic acid, resolving the latter racemic acid using (S)-(-)- α -methylbenzylamine to give the optically pure compound (2R,3R)-3-[(2-amino-phenyl)thio]-2-hydroxy-3-(4-methoxyphenyl)propionic acid, cyclizing the latter compound to give (2R,3R)-2,3-dihydro-3-hydroxy-2-(4-methoxyphenyl)-1,5-benzo-thiazepin-4(5H)-one, which is then alkylated with benzyl (2-chloroethyl) methylcarbamate to give (2R,3R)-5-[2-[N-benzyloxycarbonyl)-N-methylamino]ethyl]-2,3-dihydro-3-hydroxy-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5H)-one, and finally deprotecting the latter compound into the free amine form (2R,3R)-2,3-dihydro-3-hydroxy-2-(4-methoxyphenyl)-5-[2-(methylamino)ethyl]-1,5-benzothiazepin-4(5H)-one, which is optionally transformed into its hydrochloride.

In accordance with a broader aspect, the invention relates generally to compounds of the general formula:



wherein:

R_1 represents hydrogen or methyl,

R_2 represents hydrogen or acetyl,

R_3 represents hydrogen or methyl, and

R_4 represents hydrogen or a halogen,

with the proviso that R_1 cannot be methyl, when R_2 = acetyl,
 R_3 = methyl and R_4 = hydrogen.

These compounds can be used similarly as protectazem in providing myocardial protection in a patient during ischemia and

reperfusion and may be prepared by a similar process as the one defined above for protectazem.

MODES FOR CARRYING THE INVENTION

- 5 The invention is illustrated but not limited by the specific preparation of protectazem given below with an accompanying flow chart depicting the various reactions involved.

Methyl 3-[(2-aminophenyl)thio]-2-hydroxy-3-(4-methoxyphenyl) propionate (2).

- 10 Dissolve methyl 3-(4-methoxyphenyl)glycidate 1 (246.1 g, 1.182 mol) in toluene (2.2 L) and add 2-aminothiophenol (148.0 g, 1.182 mol). Reflux under nitrogen for 6 h and concentrate the solution to 1/3 volume. Allow the mixture to cool and add a solution of 50% hexanes/ether (100 mL). Let stand overnight to precipitate. Filter, wash the solid with 70% hexanes/ether and dry *in vacuo* to obtain the title
15 compound 2 (295.6 g, 75%). m.p. 91-93°C.

3-[(2-Aminophenyl)thio]-2-hydroxy-3-(4-methoxyphenyl)propionic acid (3).

- Dissolve the aminoester 2 (198.3 g, 594.6 mmol) in methanol (300 mL) and heat to reflux. Cautiously add a hot solution of sodium
20 hydroxide (26.72 g, 1.1 equiv.) in water (300 mL). Reflux for 20 min. and concentrate *in vacuo*. Add water (300 mL) and wash with chloroform (3x50 mL). Separate the aqueous phase and neutralize with 10% HCl to pH 5.0 (pH meter). Filter and wash the solid with water then with cold ethanol. Dry *in vacuo* at 40°C to obtain the racemic acid
25 3 (161.5 g, 85%). m.p. 174- 176°C.

(S)- α -Methylbenzylammonium (2R,3R)-3-[(2-aminophenyl)thio]-2-hydroxy-3-(4-methoxyphenyl) propionate (4).

- Dissolve the racemic acid 3 (81.7 g, 255.8 mmol) in 1 N sodium hydroxide (261 mL, 1.02 equiv) and add water (174 mL). Heat
30 the mixture to reflux.

Dissolve (S)-(-)- α -methylbenzylamine (14.6 g, 0.47 equiv) in 1 N HCl (122 mL, 0.48 equiv) and heat to about 80°C.

- Add the second solution to the first and reflux for 2 min. Cool the mixture to room temperature and then allow to stand at 0°C for
35 2 h. Collect the crystals by filtration and wash with cold water. Recrystallize with 50% aqueous ethanol to obtain the pure title

compound 4 (45.1 g, 40%). m.p. 159-160°C. $[\alpha]_D^{23}$ -463.8° (1.3, DMF).

(2R,3R)-3-[(2-Aminophenyl)thio]-2-hydroxy-3-(4-methoxyphenyl)propionic acid (5).

5 Dissolve the (-)-salt 4 (42.85 g, 97.26 mmol) in boiling water (430 mL). Add hot 1 N HCl (98 mL). Reflux for 1 min. Cool the mixture in an ice-water bath and stir vigorously to precipitate. Check (and adjust if necessary) the pH of the solution to 5.0 (pH meter). Collect the solid by filtration. Wash with water and dry *in vacuo* at 40°C
10 to obtain the title compound 5 (24.85 g, 80%). m.p. 175-176°C. $[\alpha]_D^{23}$ -362.8° (1.2 DMF).

(2R,3R)-2,3-Dihydro-3-hydroxy-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5H)-one (6)^[12].

15 Add *p*-toluenesulfonic acid (974 mg) to a mixture of the (-)-acid 5 (32.7 g, 102.4 mmol) and xylenes (400 mL). Reflux under nitrogen for 16 h, using a Dean-Stark trap to remove water. Cool the reaction mixture to 0°C to precipitate. Filter and wash the solid with a cold solution of 80% hexanes-ether. Recrystallize in isopropyl alcohol to obtain the pure title compound 6 (24.42 g, 86%). m.p. 203-205°C. $[\alpha]_D^{23}$ -56.0°(0.22, CHCl₃); -112.2° (1.15, DMF).
20

(2R,3R)-5-[2-[N-(benzyloxycarbonyl)-N-methylamino]-ethyl]-2,3-dihydro-3-hydroxy-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5H)-one (8).

25 Wash potassium hydride (35% in oil, 10.6 g) with pentane and add anhydrous DMF (330 mL). Add the solid (-)-lactam 6 (26.53 g, 88.02 mmol) by portions to allow a steady evolution of hydrogen. Stir for 5 min. after addition.

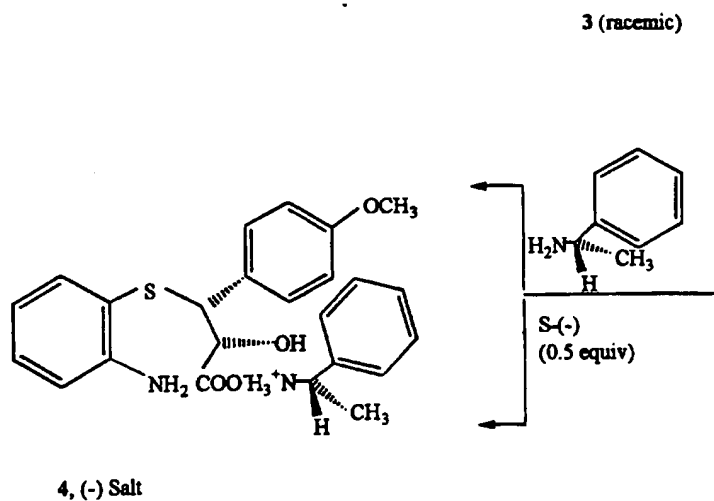
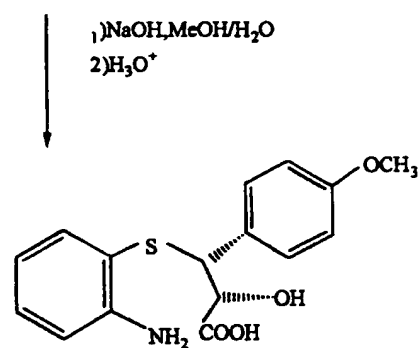
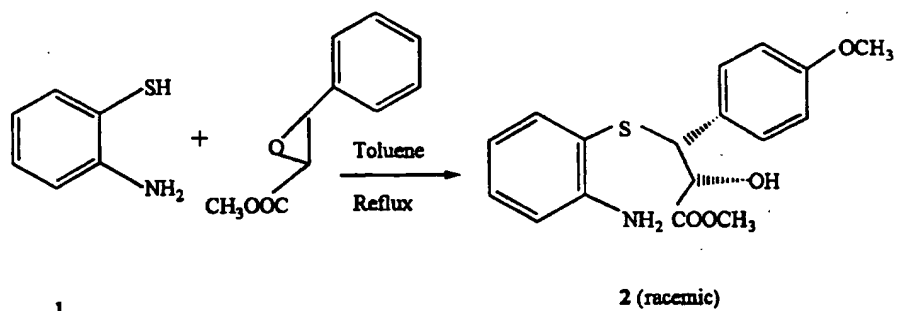
30 Add benzyl (2-chloroethyl)methylcarbamate 7^[13] (24.1 g, 1.2 equiv.) to the above reaction mixture. Rinse the container with DMF (20 mL) and add to the reaction mixture. Stir at 60°C for 18 h. Concentrate *in vacuo*. Partition the residue between ethyl acetate and water. Wash the organic phase with water, dry (MgSO₄) and evaporate the solvent *in vacuo*. Purify by silica gel flash chromatography (20:80 to 100:0 ether/hexanes) to obtain the title compound 8 (32.48 g, 75%) as a
35 resinous material.

(2R,3R)2,3-Dihydro-3-hydroxy-2-(4-methoxy-phenyl)-5-[2-(methylamino)ethyl]-1,5-benzo-thiazepin-4(5H)-one (9) and HCl salt (10).

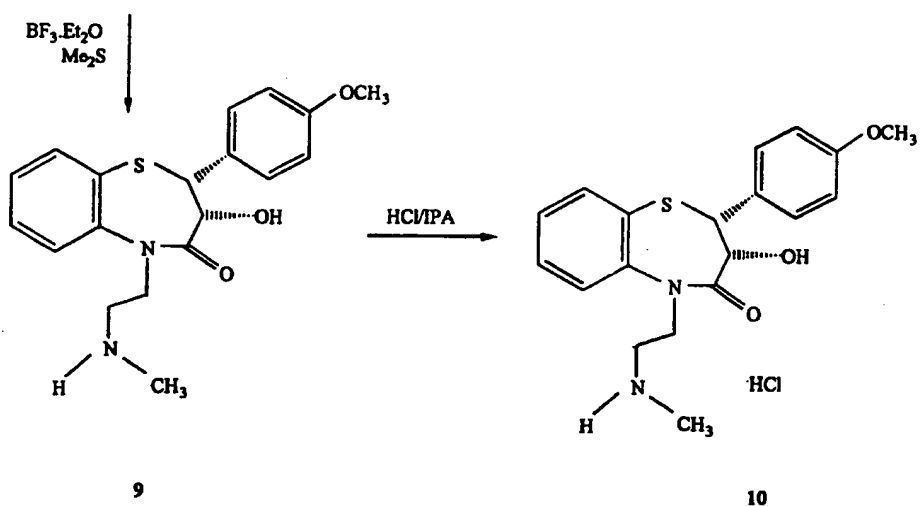
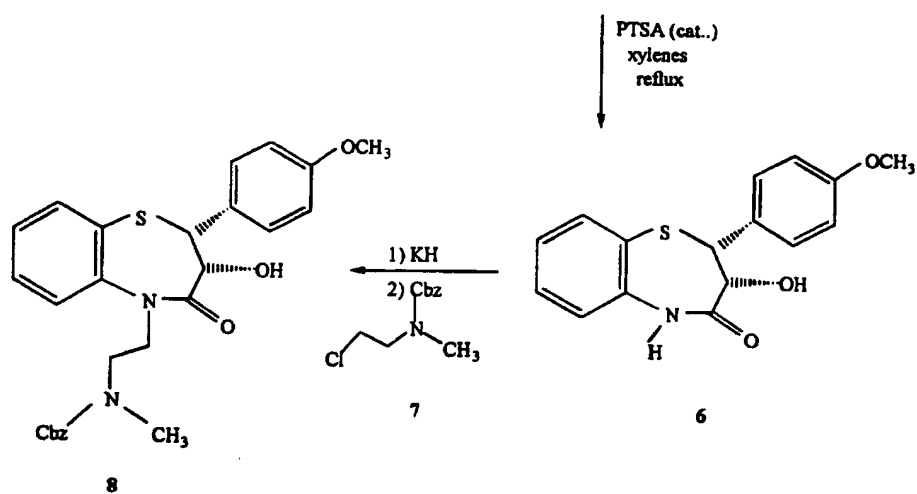
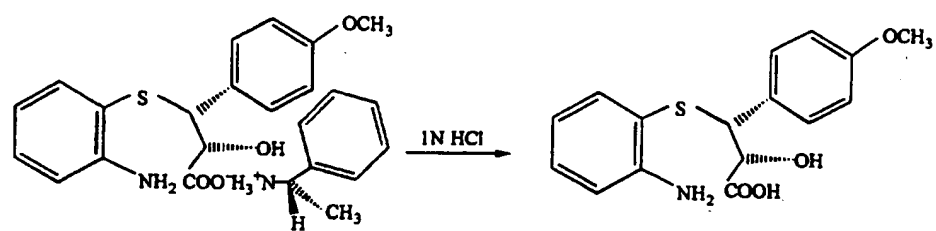
5 Dissolve 8 (25.94 g, 52.7 mmol) in dichloromethane (105 mL) and add dimethyl sulfide (104.5 mL, 27 equiv). Slowly add $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (62.9 mL, 9.7 equiv) with vigorous stirring. After 1 h add another portion of dimethyl sulfide (85 mL, 22 equiv) and stir for another 2 h. Pour the reaction mixture into an ice-cold solution of 5% NH_4OH .

10 Adjust the pH to 8. Add dichloromethane to extract (3x150 mL). Combine the organic phases and wash with brine. Dry (MgSO_4) and evaporate the solvent *in vacuo* to obtain the title compound 9.

15 Dissolve 9 in ethyl acetate (50 mL) and cool to 0°C. Add a solution of 6.8 N HCl/isopropyl alcohol (8.5 mL, 1.1 equiv) dropwise. Stir 10 min. at 0°C to complete the reaction. Add methanol (8 mL) and heat to reflux to dissolve the precipitate. Cool slowly to room temperature then let stand at 0°C to recrystallize. Filter and wash the solid with ethyl acetate. Dry *in vacuo* to obtain the pure title compound 10 (13.59 g, 65%). m.p. 208-209°C. $[\alpha]_{\text{D}}^{23} -122.8^\circ$ (0.5, H_2O).



Acid recovery
(non-resolved)



BRIEF DESCRIPTION OF DRAWINGS

FIGURE 1 is a graph showing variations of heart rate with incremental cumulative doses of protectazem;

5 FIGURE 2 is a graph showing variations in systolic blood pressure with incremental cumulative doses;

FIGURE 3 is a graph showing variations of diastolic blood pressure with incremental cumulative doses;

10 FIGURE 4 is a graph showing variations in mean arterial pressure with incremental cumulative doses;

FIGURE 5 is a graph showing variations in peak left ventricular pressure with incremental cumulative doses;

FIGURE 6 is a graph showing variations in left ventricular telediastolic pressure with incremental cumulative doses;

15 FIGURE 7 is a graph showing variations in dP/dT max with incremental cumulative doses;

FIGURE 8 is a graph showing variations in LAD blood flow with incremental cumulative doses;

20 FIGURE 9 is a graph showing variations in pressure-rate with incremental cumulative doses;

FIGURE 10 is a graph showing variations in infarct size (% area at risk) with different doses;

FIGURE 11 is a graph of the effect of time treatment versus occlusion with different doses on infarct size in rabbits; and

25 FIGURE 12 is a graph showing the effect of the treatment on infarct size in the dog.

EXPERIMENTAL STUDIES

1. Dose-related cardiovascular effects (Figs. 1 to 11):

Anesthetized open chest dogs were given at 15 min. intervals incremental i.v. doses of protectazem from 400 to 6400 µg/kg. Heart rate (Fig. 1), arterial systolic (Fig. 2), diastolic (Fig. 3) and mean blood pressure (Fig. 4) were monitored. Systolic ventricular (Fig. 5) and telediastolic (Fig. 6) pressures were monitored along with dP/dT (Fig. 7). Left descending coronary artery (LAD) blood flow (Fig. 8) was also measured using an electromagnetic flow probe implanted on the left anterior descending coronary artery after the first marginal branch. The pressure rate-product (systolic pressure x heart rate /100; Fig. 9) was used as the index of cardiac work load or metabolic demand.

It can be observed that protectazem had no effect on heart rate and slight hypotensive effects only at very high dosages. Myocardial contractility was unchanged as indicated by dP/dT max which was not modified by any dosage. Cardiac work load and LAD coronary blood flow were also not modified by the drug.

2. Cardioprotection in the rabbit:

Ischemia and reperfusion. A left thoracotomy was performed in rabbits and the left coronary artery occluded for 40 min. followed by 6 hours of reperfusion. Infarct size was estimated by using triphenyltetrazolium (TTC) staining after perfusing monastral blue to delimit the ischemic area at risk. Infarct size was quantified in percent necrotic area over the area at risk by planimetry after sectioning the left ventricle in 2 mm slices.

Treatment groups. After 30 min. coronary occlusion or 10 min. before reperfusion, a bolus i.v. injection of the agent was followed by a perfusion throughout reperfusion. Controls received physiologic saline. Doses were either 100 µg/kg bolus + perfusion of 1 µg/kg/min.. or multiples (2x pr 4x) of this dosage. Results are presented in Figure 10.

In a second series of experiments, the dosage of 400 µg/kg bolus + 4 µg/kg/min.. perfusion was used. Treatments were started either before or after 15, 30 and 37 min. of ischemia (Fig. 11).

Results:

It can be observed that a dosage of 400 $\mu\text{g/kg}$ produced a 60% reduction of infarct size ($P < 0.05$). Doses of 200 and 100 $\mu\text{g/kg}$ were less effective or inactive (Fig. 10).

In series 2 experiments (Fig. 11), injecting the drug at 400 $\mu\text{g/kg}$ (bolus) + perfusing 4 $\mu\text{g/kg/min.}$, before or after 15 min. or 30 min. coronary occlusion limited infarct size by about 50% ($P < 0.05$). However, administering the drug 3 min. before reperfusion did not reduce infarct size, suggesting that the drug must be given before reperfusion and must reach the ischemic territory in these severely ischemic preparations virtually devoid of collateral flow, to be effective.

3. Cardioprotection in the dog.

Ischemia and reperfusion. Mongrel dogs of either sex weighing 18 to 28 kg were anesthetized with sodium pentobarbital (30 mg/kg, i.v.), intubated and ventilated mechanically with room air. Lead II electrocardiogram was monitored, and catheters were implanted in the femoral artery and vein. After injecting pancuronium bromide (0.1 mg/kg), a left thoracotomy was performed at the fifth intercostal space, and the heart was suspended in a pericardial cradle. A catheter was placed in the left ventricle for monitoring left ventricular pressure. Another catheter was located into the left atrium for microsphere injection and blood sampling.

The LAD was isolated distal to the first diagonal branch, and an electromagnetic flow probe was installed to measure coronary blood flow. Five min. before occlusion, a micrometer occluder was adjusted to produce a critical stenosis. The stenosis was sufficient to prevent the hyperemic response produced after release of a 10-sec occlusion of the coronary. The LAD was then occluded for 90 min. and reperfused for 6 hours with the critical stenosis left in place, to mimic the commonly encountered clinical situation after coronary thrombolysis for acute myocardial infarction.

Experimental design. Animals were assigned at random between 3 groups. Controls received saline and treated dogs were given one of the following treatments: 400 $\mu\text{g/kg}$ i.v. + perfusion of 4 $\mu\text{g/kg/min.}$, at 10 min. before reperfusion or 800 $\mu\text{g/kg}$ i.v. + perfusion of 8 $\mu\text{g/kg/min.}$ at 15 min. after coronary occlusion.

Infarct sizing. After 6 hours of reperfusion, heparin (10,000 units) was injected i.v., and the animals were sacrificed with an overdose of pentobarbital. The heart was excised rapidly, and the site of occlusion on the LAD and the aorta above the coronary ostia were cannulated and perfused with saline (0.9%) for 5 min.; subsequently, the aorta was perfused with Evans blue (Sigma Co., St. Louis, MO) (0.5% in saline), and the LAD with saline 0.9%, at a constant pressure of 100 mm Hg for 5 min. The left heart was then embedded in a polyurethane foam, and cut with a commercial meat slicer into 7-mm-thick transverse slices. The slices were weighed and immersed into triphenyltetrazolium chloride (TTC) for 10 min. (37°C). The normally perfused myocardium (Evans blue positive), the area at risk (Evans blue negative) and the necrotic myocardium (Evans blue and TTC negatives) were delineated, and respective areas were estimated on each slice by computed planimetry. The area at risk was expressed in percentage of the left ventricle, and infarct size was expressed in percentage of both left ventricle and the area at risk after summing values from all slices.

Regional myocardial blood flow. Regional myocardial blood flow was estimated with the reference withdrawal method using 15- μ m diameter microspheres labeled with ^{46}Sc . Approximately 3 million microspheres, adequately dispersed in 1 mL of suspending medium containing 0.01% Tween 80®, were injected into the left atrium over 20 sec and flushed with 20 mL of saline. A reference arterial blood sample was collected from the aortic catheter at a constant rate for 150 sec, starting 15 sec before each microsphere injection. Regional blood flow was estimated after 15 min. occlusion for assessment of collateral flow.

For regional myocardial blood flow analysis, four central left ventricular slices were dissected into ischemic and nonischemic parts. Sections from the central ischemic and from the non-ischemic wall opposite to the area risk were isolated and divided further into three equal subendocardial, midventricular and subepicardial portions. Each sample was weighed and counted along with reference samples in a gamma counter with selected energy windows. After correcting for background and spillover between isotopes, blood flow was estimated and expressed in mL of blood/min. per g of tissue.

Results:

Figure 12 illustrates the relationship between infarct size and collateral flow in the 3 study groups.

It can be observed that for a similar degree of ischemia or collateral blood flow, treated dogs developed smaller infarcts than controls, as confirmed by covariance analysis. Mean reductions of infarct size were greater than 40% with treatment at 10 min. before reperfusion.

It has therefore been established that protectazem and its derivatives are potent cardioprotective agents.

The substance tested exerts no cardiovascular depressant activity limiting its use in the set up of acute myocardial infarction. It has no direct coronary vasodilator activity. It is active rapidly at low dosages after i.v. injection and is effective before, during ischemia-reperfusion. It is a highly water soluble agent.

It is believed that the compounds according to the invention have a unique cardioprotective activity profile. They constitute a new type of drug for cardioprotection offering high potentials for future clinical use in the set up of coronary thrombolysis in acute myocardial infarction.

The compounds according to the invention may also be beneficial in several other clinical conditions such as treatment of stable and unstable angina, non-Q-wave myocardial infarction, peripheral and cerebral trauma ischemia and reperfusion, organ graft preservation, etc.

Although the invention has been specifically described with reference to one compound species and one specific use, it is understood that it is susceptible to broad applications without departing from the scope and spirit of the invention.

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J. Pharmacol. Exp. Ther. 268: 1252-1260, 1994.
- 30 9. Roberts R.
Thrombolysis and its sequelae. Calcium antagonist as
potential ajunctive therapy.
Circulation 80 (suppl.IV): 93-101, 1989.
10. Messerli F.H., Weiner D.A.
35 Are all calcium antagonists equally effective for reducing
infarction rate?

Am. J. Cardiol. 72: 818-20, 1993.

II. Rousseau G., Hébert D., Libersan D., Khalil A., St-Jean G., Latour J.G.

5 Importance of platelets in myocardial injury after reperfusion
in the presence of residual coronary stenosis in dogs.

Am. Heart J. 125: 1553-63, 1993.

12. According to the procedure of A.Schwartz described for the (2S,3S)-
isomer of 6:

10 Schwartz, A., Madan, P.B., Mohacsi, E., O'Brien, J.P.,
Todaro L.J., and Coffen, D.L. Enantioselectives Synthesis of
Calcium Channel Blockers of the Diltiazem Group. J. Org.
Chem. 57, 851-856, 1992.

13. Li, R.; Farmer, P.S. Xie, M. Quilliam, M.A.

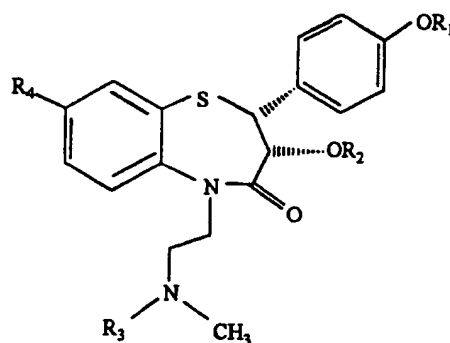
15 Pleasance, S. Howlett, S.E. and Yeung, P.K.F.

Synthesis, Characterization, and Ca^{2+} Antagonist Activity of
Diltiazem Metabolites.

J. Med. Chem. 35, 3246-3253, 1992.

CLAIMS

1. Method of providing myocardial protection in a patient during ischemia and reperfusion which comprises administering to said patient an effective amount of a compound of formula:



(2R,3R)

wherein

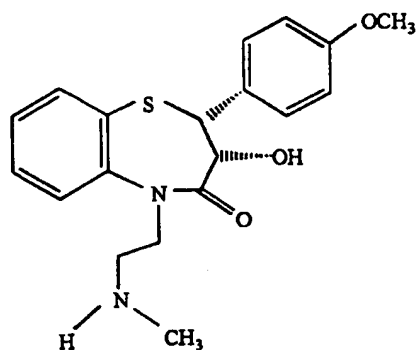
- 10 R_1 represents hydrogen or methyl,
 R_2 represents hydrogen or acetyl,
 R_3 represents hydrogen or methyl, and
 R_4 represents hydrogen or a halogen,
 salts or derivatives thereof,

- 15 with the proviso that R_1 cannot be methyl when $R_2 = \text{acetyl}$,
 $R_3 = \text{methyl}$ and $R_4 = \text{hydrogen}$.

2. Method according to claim 1, wherein said compound is administered before or after coronary occlusion up to a few minutes before and during coronary reperfusion.

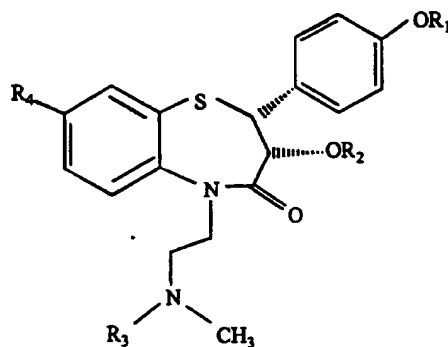
3. Method according to claim 2, wherein said compound is administered intravenously at doses of about 20 to about 200 mg or more.

4. Method according to claim 3, wherein said compound has the formula:

(2*R*,3*R*)

or a salt thereof.

5. Compounds of the general formula:

(2*R*,3*R*)

wherein

R_1 represents hydrogen or methyl,

R_2 represents hydrogen or acetyl,

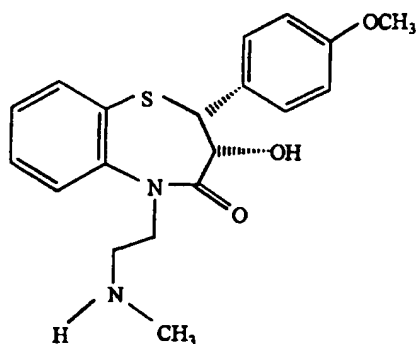
R_3 represents hydrogen or methyl, and

R_4 represents hydrogen or a halogen,

with the proviso that R_1 cannot be methyl when R_2 = acetyl,

R_3 = methyl and R_4 = hydrogen.

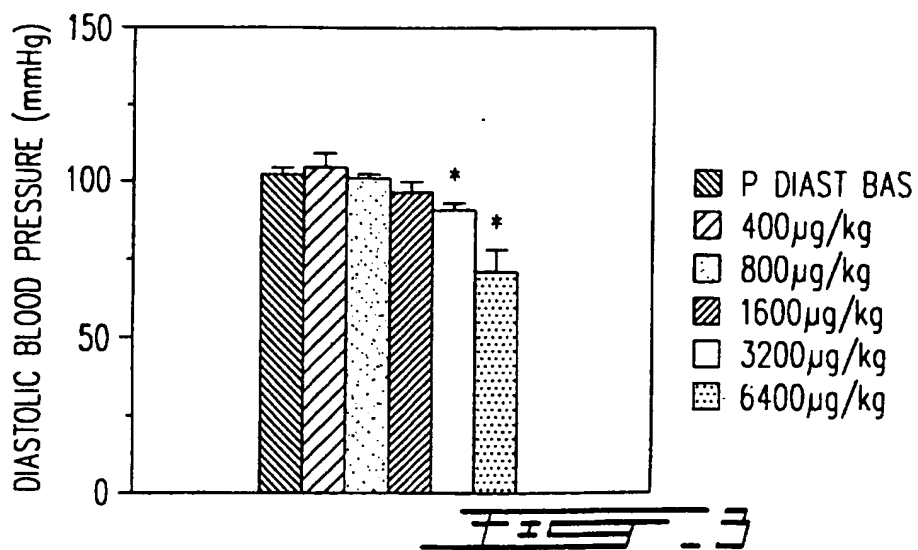
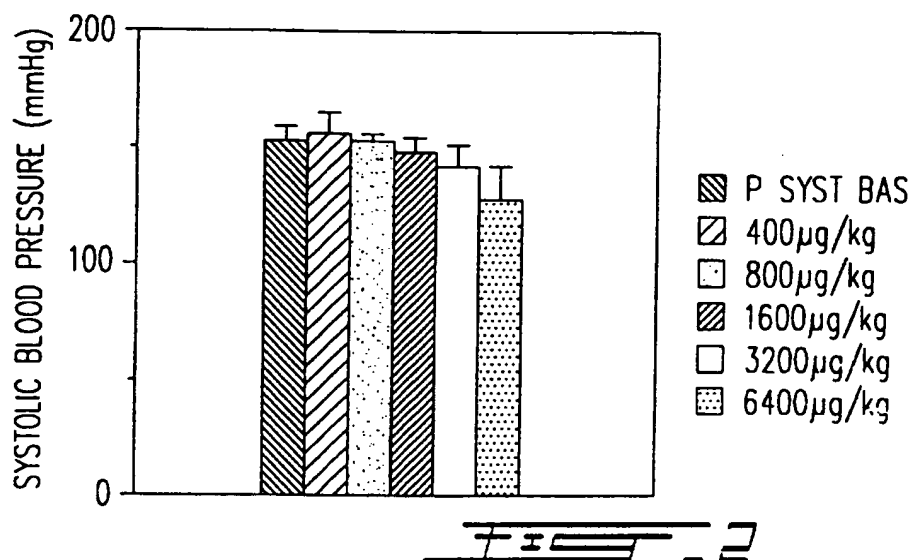
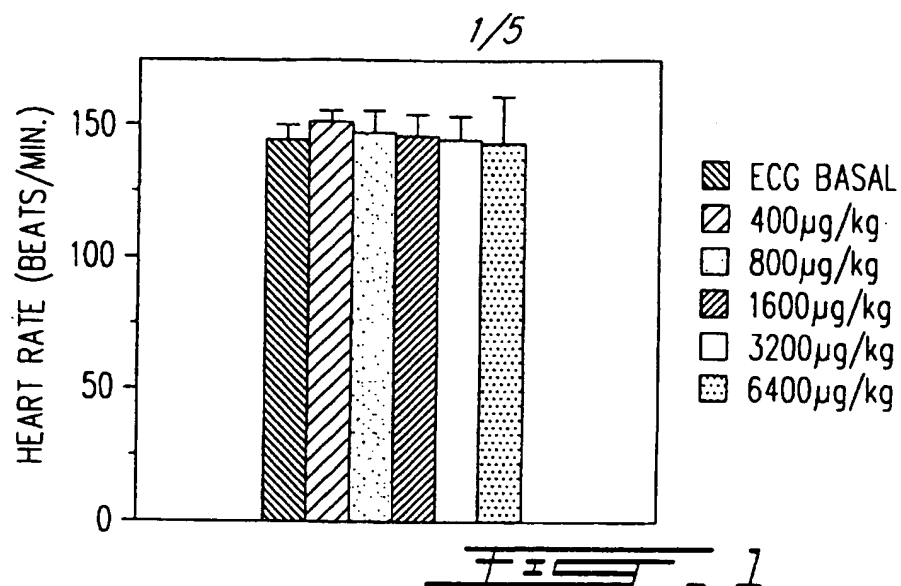
6. The compound of the formula:

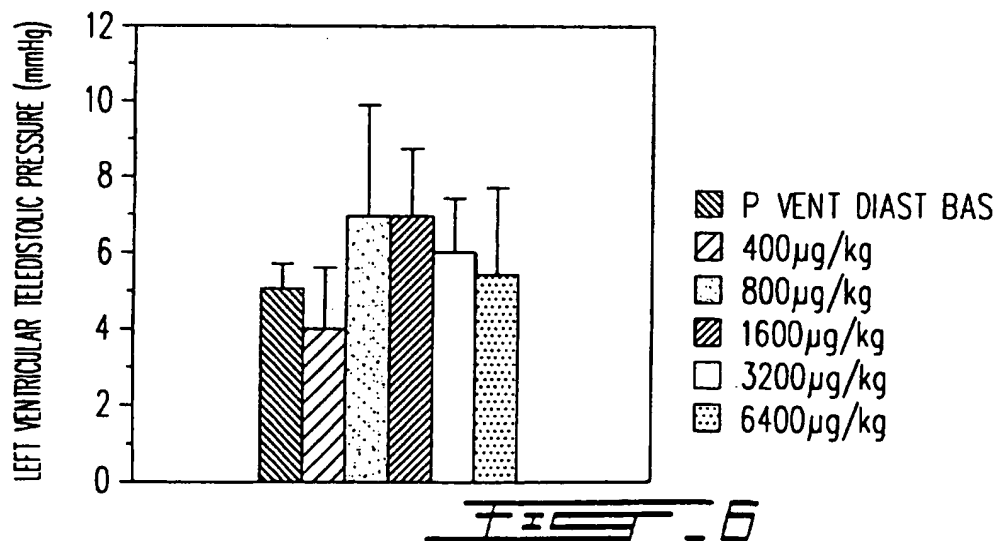
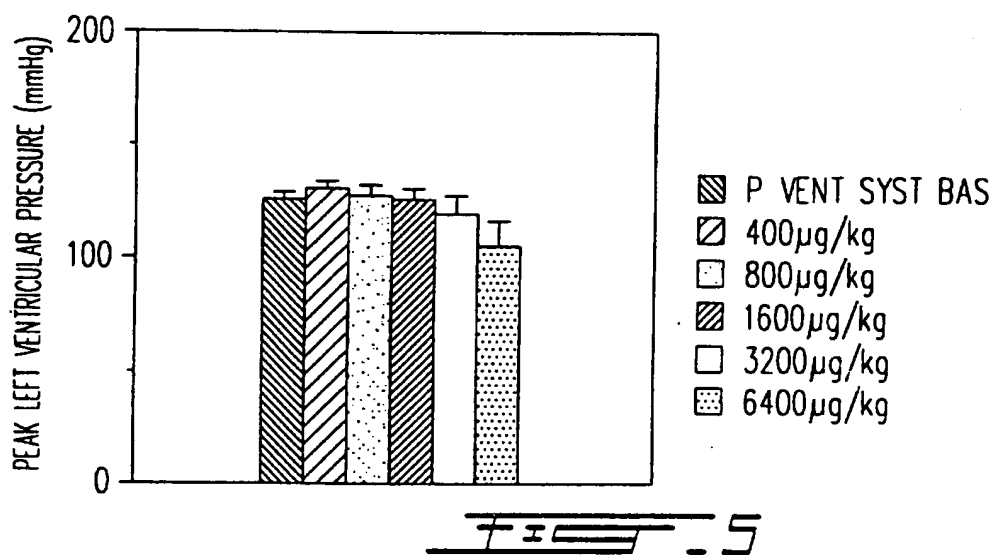
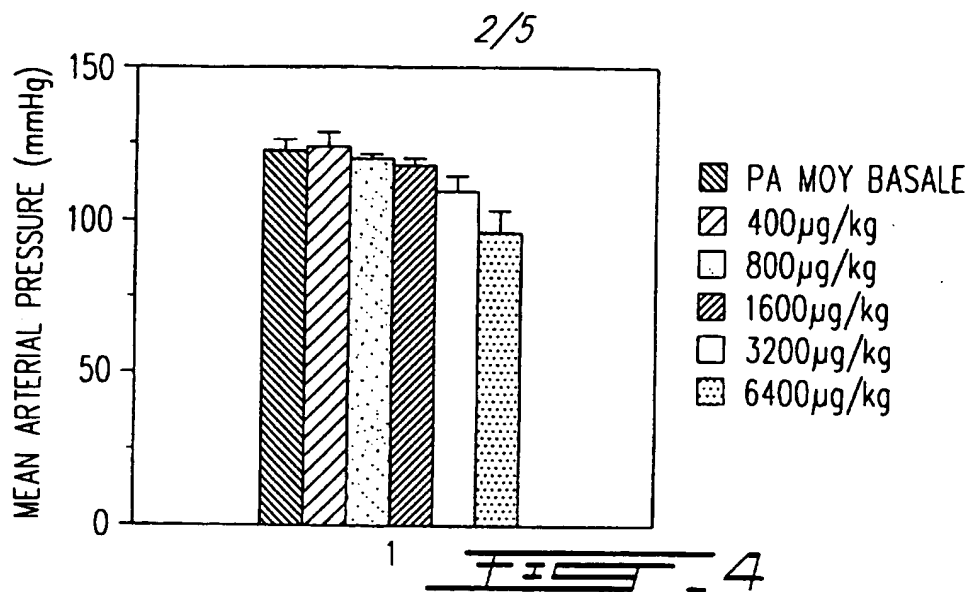


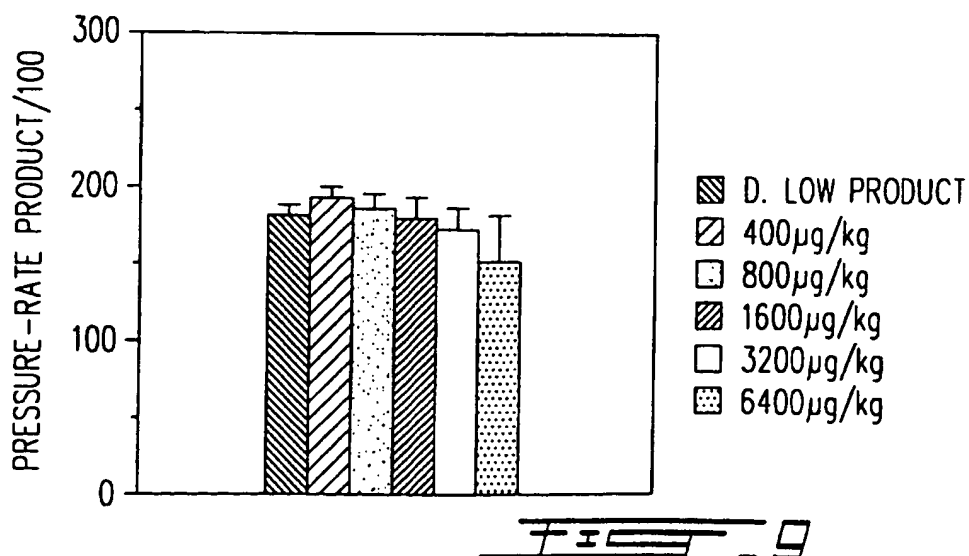
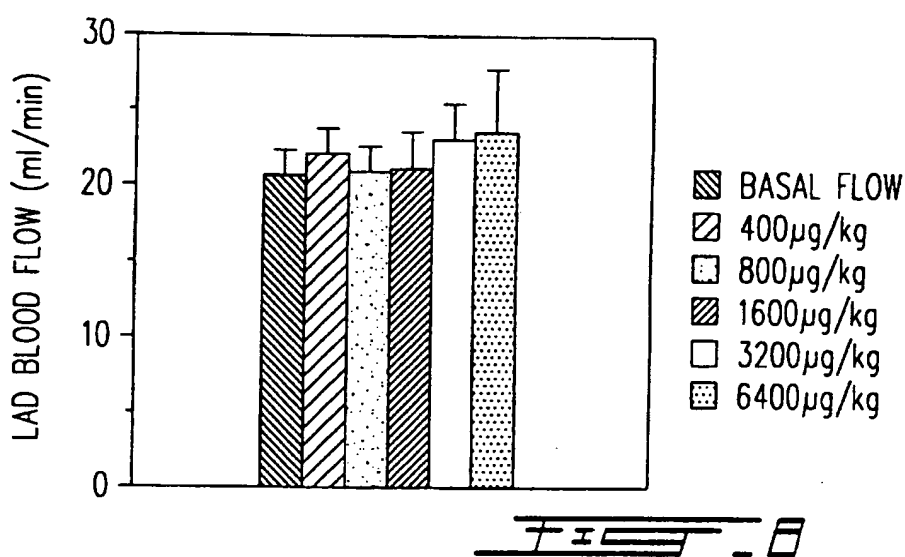
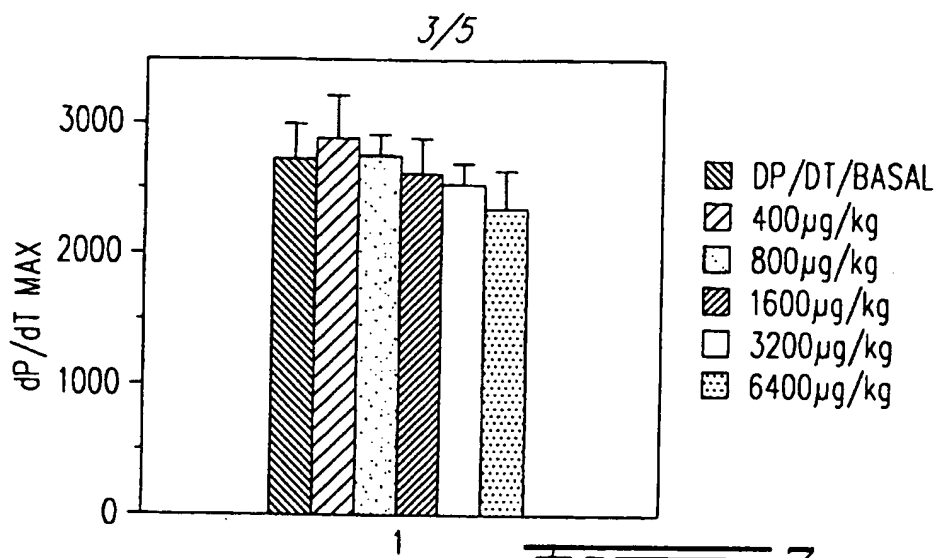
(2R,3R)

the HCl salt thereof having the following physical characteristics:
m.p. 208-209°C. $[\alpha]_{D23} - 122.8^\circ$ (0.5, H₂O), or a salt thereof.

- 5 7. Process which comprises condensing *trans*- methyl 3-(4-methoxyphenyl)glycidate with 2-amino-thiophenol to give the compound methyl 3-[(2-aminophenyl)thio]-2-hydroxy-3-(4-methoxyphenyl) propionate in its racemic form, saponifying the latter
10 with methanolic aqueous sodium hydroxide to give the corresponding racemic acid 3-[(2-aminophenyl)thio]-2-hydroxy-3-(4-methoxyphenyl) propionic acid, resolving the latter racemic acid using (S)-(-)- α -methylbenzylamine to give the optically pure compound (2R,3R)-3-[(2-amino-phenyl)thio]-2-hydroxy-3-(4-methoxyphenyl)propionic acid,
15 cyclizing the latter compound to give (2R,3R)-2,3-dihydro-3-hydroxy-2-(4-methoxyphenyl)-1,5-benzo-thiazepin-4(5H)-one, which is then alkylated with benzyl (2-chloroethyl)methylcarbamate to give (2R,3R)-5-[2-[N-benzyloxycarbonyl)-N-methylamino]ethyl]-2,3-dihydro-3-hydroxy-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5H)-one, and
20 finally deprotecting the latter compound into the free amine form (2R,3R)-2,3-dihydro-3-hydroxy-2-(4-methoxyphenyl)-5-[2-(methylamino)ethyl]-1,5-benzothiazepin-4(5H)-one, which is optionally transformed into its hydrochloride.







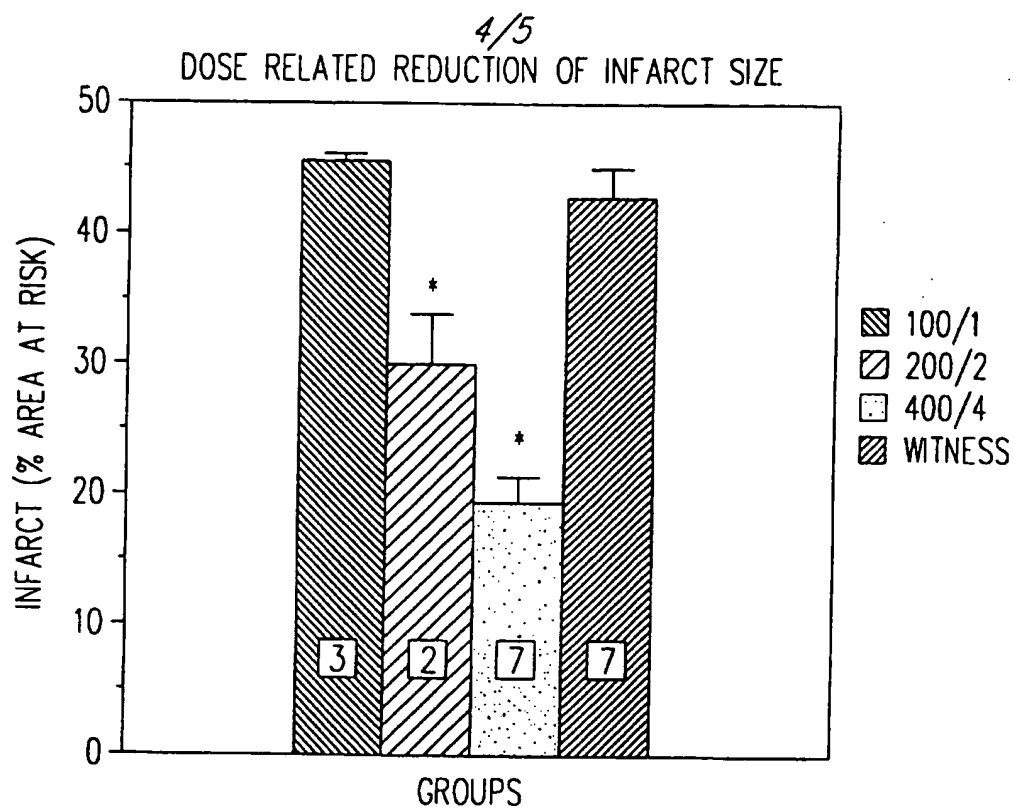


FIG 10

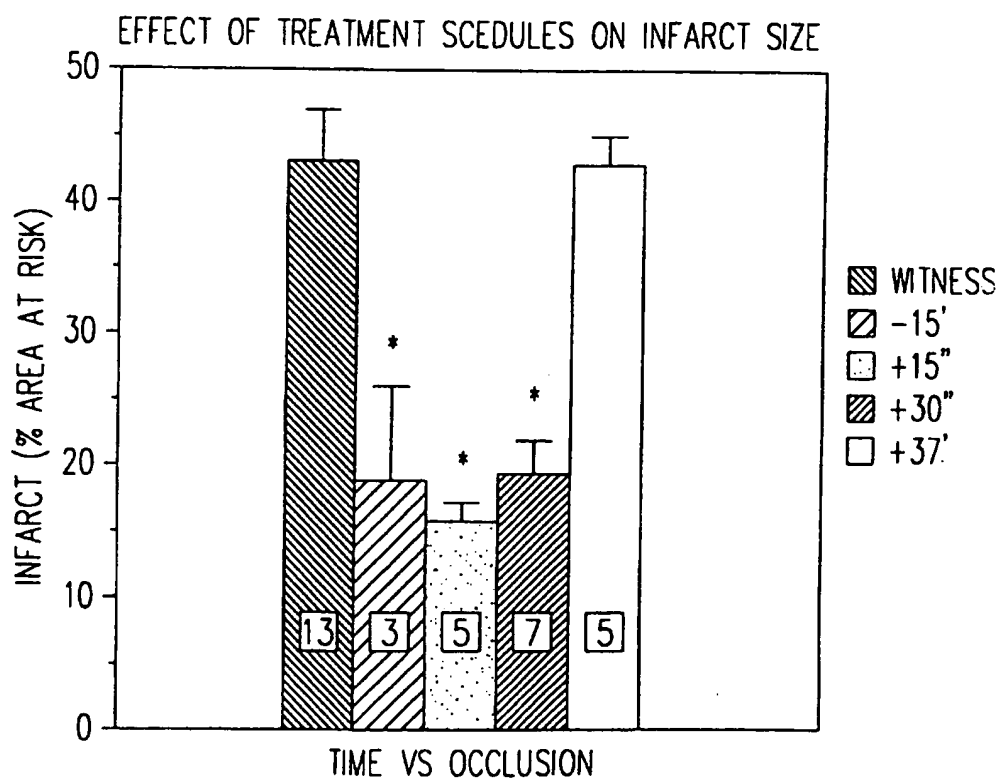
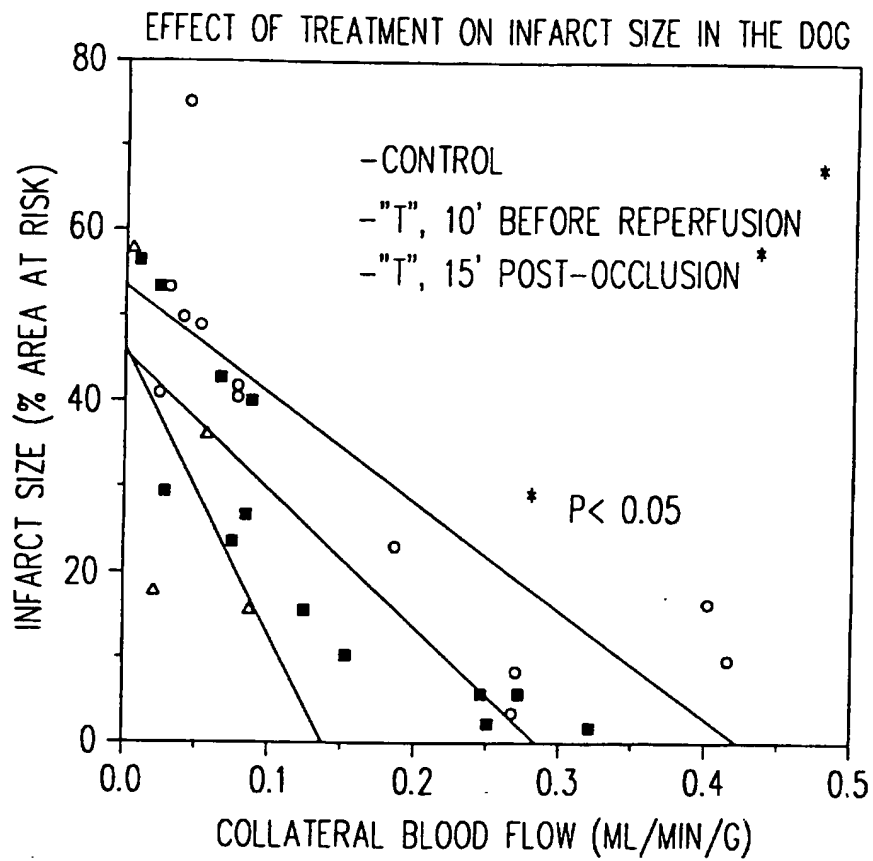


FIG 11

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FIG-12

INTERNATIONAL SEARCH REPORT

International Application No

PCT/CA 95/00514

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/55 C07D281/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP,A,0 158 339 (TANABE SEIYAKU CO., LTD.) 16 October 1985 see the whole document ---	5-7
X	HELVETICA CHIMICA ACTA, vol. 67, no. 3, 2 May 1984 BASEL CH, pages 916-26, B. KOJIC-PRODIC ET AL. 'Absolute conformation and configuration of (2S,3S)-acetoxy-5-(dimethylaminoethyl)-2- (4-methoxyphenyl)-2,3-dihydro-1,5- benzothiazepin-4(5H)-one chloride (diltiazem hydrochloride)' see the whole document, particularly page 918, compound (-)-7 --- -/--	5-7



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
E earlier document but published on or after the international filing date
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
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X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
& document member of the same patent family

Date of the actual completion of the international search

21 December 1995

Date of mailing of the international search report

04.01.96

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
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Fax (+ 31-70) 340-3016

Authorized officer

Allard, M

INTERNATIONAL SEARCH REPORT

Int. Application No
PCT/CA 95/00514

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOURNAL OF MEDICINAL CHEMISTRY, vol. 34, no. 2, February 1991 WASHINGTON DC, US, pages 675-87, H. INOUE ET AL. 'Synthesis of halogen-substituted 1,5-benzothiazepine derivatives and their vasodilating and hypotensive activities' see the whole document ---	5-7
X	CHEMICAL ABSTRACTS, vol. 100, no. 5, 30 January 1984 Columbus, Ohio, US; abstract no. 34572m, page 456; see abstract & JP,A,58 113 185 (HAMARI YAKUHI KOGYO CO., LTD.) & 'Chem. Abs. 11th Coll. Chem. Substance Index' page 11859CS, right-hand column, lines 37 and 41 from bottom ---	5-7
X	CHEMICAL ABSTRACTS, vol. 108, no. 7, 15 February 1988 Columbus, Ohio, US; abstract no. 56129q, page 733; see abstract & JP,A,62 161 776 (TANABE SEIYAKU CO., LTD.) ---	5-7
X,Y	CHEMICAL ABSTRACTS, vol. 99, no. 7, 15 August 1983 Columbus, Ohio, US; abstract no. 47687p, A. KIYOMOTO ET AL. 'Inhibition of platelet aggregation by diltiazem. Comparison with verapamil and nifedipine and inhibitory potencies of diltiazem metabolites' page 32; see abstract & CIRC. RES., SUPPL., vol. 52, no. 1, 1983 pages 115-19, ---	5-7

-/--

INTERNATIONAL SEARCH REPORT

International Application No

PCT/CA 95/00514

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JOURNAL OF MEDICINAL CHEMISTRY, vol. 35, no. 17, 21 August 1992 WASHINGTON DC, US, pages 3246-53, R. LI ET AL. 'Synthesis, characterization, and Ca ²⁺ antagonistic activity of diltiazem metabolites' cited in the application see the whole document ---	6,7
Y	CHEMICAL ABSTRACTS, vol. 119, no. 17, 25 October 1993 Columbus, Ohio, US; abstract no. 173953s, G. ROUSSEAU ET AL. 'Sustained myocardial protection by clentiazem (TA-3090) after a 90-minute coronary occlusion and 72 hours of reperfusion in dogs with collateral flow' page 65; cited in the application see abstract & J. CARDIOVASC. PHARMACOL., vol. 22, no. 2, 1993 pages 264-72, ---	1-7
Y	CHEMICAL ABSTRACTS, vol. 114, no. 5, 4 February 1991 Columbus, Ohio, US; abstract no. 35438y, Y. NASA ET AL. 'Both d-cis- and l-cis-diltiazem have anti-ischemic action in the isolated, perfused working rat heart' page 18; see abstract & J. PHARMACOL. EXP. THER., vol. 255, no. 2, 1990 pages 680-9, -----	1-7

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA95/00514

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 1-4 are directed to a method of treatment of the human/
animal body, the search has been carried out and based on the alleged
effects of the compounds/composition.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such
an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/CA 95/00514

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-158339	16-10-85	AU-B- 572978	19-05-88
		AU-B- 4039285	17-10-85
		CA-A- 1218991	10-03-87
		JP-C- 1706316	27-10-92
		JP-B- 3074659	27-11-91
		JP-A- 60226866	12-11-85
		SU-A- 1632372	28-02-91
		SU-A- 1358784	07-12-87
		US-A- 4585768	29-04-86

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